

REMARKS

A Petition for Extension of Time is being concurrently filed with this Amendment. Thus, this Amendment is being timely filed.

Applicants respectfully request the Examiner to reconsider the present application in view of the foregoing amendments to the claims and the following remarks.

Status of the Claims

Claims 9, 12, 15, 18 and 21-30 are currently pending in the present application. Claims 9, 12, 15, 18 and 21 have been withdrawn from consideration as being directed to a non-elected invention. The Office Action is Final. Claims 22-27 and 30 were amended without prejudice or disclaimer. The amendments to claims 22-27 and 30 further define and clarify the present invention. Specifically, claims 22 and 30 were amended so that the contacting step is carried out after or simultaneously to the centrifugation step. Support can be found at page 5, lines 8-12, and Table 13 of the present specification. As shown in Table 13 the advantageous effect of present invention is also attained by carrying out the first and second steps simultaneously. Thus no new matter has been added.

Based upon the above considerations, entry of the present Amendment is respectfully requested.

37 C.F.R. § 1.132 Declaration

A 37 C.F.R. § 1.132 Declaration of Mr. Yukoh Hiei is enclosed with the instant amendment for the Examiner's consideration.

The Examiner is respectfully requested to review Mr. Hiei's enclosed Declaration (as well as the previously submitted Declaration of Mr. Hiei (*submitted on November 13, 2007*)) at this time, as it material to a consideration of whether the cited references renders obvious any of instantly pending claims 22-30.

Objections to the Claims

Claims 22, 27 and 30 were objected to due to informalities. Applicants have amended claims 22, 27 and 30 to include a comma before "wherein" as the Examiner suggested.

Applicants respectfully request reconsideration and withdrawal of the present objection.

Issue Under 35 U.S.C § 112, Second Paragraph, Indefiniteness

Claim 30 stands rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter, which Applicants regard as the invention.

The Examiner asserts that it is unclear what was intended by the recitation "maize angiosperm." Applicants respectfully traverse.

Although Applicants disagree, in order to further prosecution, Applicants amended claim 30, without prejudice or disclaimer, to further clarify the invention. Applicants respectfully requests reconsideration, and subsequent withdrawal of the present rejection.

Issue Under 35 U.S.C. § 103(a), Obviousness

Claims 22-29 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Konzak *et al.*, U.S. Patent No. 6,362,393 (hereinafter “Konzak”) in view of Forreiter *et al.*, “*Stable Transformation of an Arabidopsis Cell Suspension Culture With Firefly Luciferase Providing a Cellular System for Analysis of Chaperone Activity In Vivo*,” The Plant Cell, vol. 9, pp. 2171-2181 (1997) (Hereinafter “Forreiter”) in light of Pierce Biotechnology, Inc., Technical resource 1/2005, “*Convert between times gravity (x g) and centrifuge rotor speed (RPM)*” (Hereinafter “Pierce”).

Reconsideration and withdrawal of this rejection to the claims is respectfully requested based on the following considerations.

The Examiner asserts that Konzak discloses a method of centrifuging plant tissues of rice or corn at the acceleration of 100G for 3 minutes prior to gene introduction. The Examiner also asserts that Konzak teach that gene transformation could occur at any time of the procedure by using *Agrobacterium tumifaciens*. The Examiner also states that Konzak do not teach the centrifugation speed of 1000G to 150,000G.

The Examiner also asserts that Forreiter discloses a method of gene transfer of *Arabidopsis thaliana*, wherein the protoplasts were collected by centrifuging the cells for ten minutes at 600G and that transient transformation was performed.

Additionally, the Examiner also asserts it would have been obvious to one of ordinary skill in the art to use the method of promoting gene introduction into plant cells by centrifuging the plant cells or plant tissues before gene introduction by applying *Agrobacterium* as taught by Konzak, and to modify that method by adjusting the centrifugal acceleration as taught by Forreiter given the advantage of separating the tissues at higher speed.

Additionally the Examiner noted that Pierce discloses “centrifugation speed and time often are not critical factors in routine sampling-handling....” Applicants respectfully traverse.

Graham v. John Deere, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966), has provided the controlling framework for an obviousness analysis. A proper analysis under § 103(a) requires consideration of the four *Graham* factors of: determining the scope and content of the prior art; ascertaining the differences between the prior art and the claims that are at issue; resolving the level of ordinary skill in the pertinent art; and evaluating any evidence of secondary considerations (e.g., commercial success; unexpected results). 383 U.S. at 17, 148 USPQ at 467.

Based on a full review of the enclosed 37 C.F.R. § 1.132 Declaration of Mr. Hiei (as well as the 37 C.F.R. § 1.132 Declaration of Mr. Hiei previously submitted on November 13, 2007), Applicants believe that there is a distinction between the present invention and the cited references.

In the references cited by the Examiner, the centrifugation is carried out in order to separate single plant cells such as protoplasts and microspores. In view of this, Applicants have amended claims 22 and 30, without prejudice or disclaimer, to exclude the use of plant cells from the scope of the claims.

Applicants respectfully submit that the Examiner misunderstands that the efficiency of gene introduction is promoted purified by centrifugation. In this regard, Applicants explain below the technological significance of the present invention.

The essential features of the present invention is “centrifuging a plant sample” and “bringing the plant sample into contact with *Agrobacterium*.” The present inventors discovered an unexpected prominent effect that by these features, *i.e.*, the plant tissue is changed to a physiological state in which the genes are likely to be introduced. This discovery is surprising to those skilled in the art and is not obvious. Applicants bring to the attention of the Examiner, page 2, lines 12-14 of the present specification, which reads: “On the other hand, studies for changing the plant tissue before infection of *Agrobacterium* to a physiological state in which the genes are likely to be introduced have been scarcely made.” This clearly states the difference between the present invention and the prior art.

More particularly, the term “physiological state is changed” means that the cell division of the plant tissue to which the gene is to be introduced is activated by the centrifugation treatment so that a physiological state in which introduction of a foreign gene is likely to occur is brought about. Assuming that the plant tissue to which a gene is to be introduced is, for example, a scutellum, the cell division of the scutellum is activated by the centrifugation treatment so that the scutellum becomes larger and heavier. This is supported by the data from Figures 1 and 2 in the Declaration submitted on November 13, 2007. Figures 1 and 2 are plan views of the scutella subjected to a centrifugation treatment and not subjected to a centrifugation treatment, respectively. As can be seen from these figures, the size of each scutellum (each grain in the figures) is larger when the scutellum was subjected to the centrifugation treatment. For

example, comparing the left upper photograph in Figure 2 (not centrifuged) and the right lower photograph (2000G), the size of each scutellum in the right lower photograph is apparently larger than that in the left upper photograph.

To further substantiate this phenomenon, Applicants herein submit another 37 C.F.R. § 1.132 Declaration. Figure 1 of the new Declaration compares the weight of the scutella excluding hypocotyl, which scutella were subjected to a centrifugation treatment and not subjected to a centrifugation treatment, respectively. It can be clearly seen that the scutella subjected to the centrifugation treatment is heavier than those not subjected to the centrifugation treatment. Further, Figure 2 of this new Declaration shows the colors of the scutella. Apparently, the color is different between those subjected to the centrifugation treatment and those not subjected to the centrifugation treatment. Thus, it can be seen that the physiological state was changed by the centrifugation treatment. A black and white copy of the Declaration is attached.

Thus, since the present invention is a technique in which the “physiological state” is “changed” by a centrifugation treatment under prescribed conditions, even if only one plant tissue is subjected to the centrifugation treatment, the effect is obtained.

Thus, it is apparent that the present invention is different from the technique by which a tissue is purified from the state in which plant fragments exist as a mixture. Therefore, the present invention is not obvious over the cited references.

Such facts set forth in the enclosed 37 C.F.R. § 1.132 Declaration of Mr. Hiei evidence the non-obviousness of the instant invention as recited in pending claims 22-30 over the disclosure of the cited references. Any contentions of the USPTO to the contrary are not

sustainable and therefore must be reconsidered at present.

Additionally, the Examiner alleges that the Tables 1-3 do not show consistent results (page 8, line 11 of the Final Office Action). Applicants respectfully submit that this is not correct. The present invention is based on the discovery that the physiological state of a desired plant tissue to which a gene is to be introduced is changed to the state in which the gene introduction is likely to occur by centrifugation, especially by centrifugation with an acceleration of not less than 1000G. Both of the immature embryos and *Agrobacterium* used in the Example are biological materials. Therefore, when 10 immature embryos are used as the samples, for example, it is impossible to obtain 10 immature embryos having the exact same properties. The Example's goal was to show the technical aspects of the present invention through experimental data, so that data obtained using different media are also shown. However, as a whole, the tendency of the effect of the present invention is sufficiently shown. By also referring to the previously filed Declaration submitted on November 13, 2007, the effect of the present invention and its critical significance are clearer. If the Examiner requires, Applicants can present additional experimental data further supporting the effect of the present invention. From the above discussion, the results shown in Tables 1 to 3 support the present invention, and are consistent.

In light of the above remarks, and the enclosed 37 C.F.R. § 1.132 Declaration, no teaching, disclosure, reason or rationale is provided in the cited references that would allow one of ordinary skill in the art to arrive at the instant invention as claimed, it follows that the same references are incapable of rendering the instant invention obvious under the provisions of 35 USC § 103(a).

Applicant respectfully requests reconsideration and withdrawal of the present rejection.

In view of the above, Applicants believe the pending application is in condition for allowance.

CONCLUSION

In view of the above remarks, it is believed that claims are allowable.

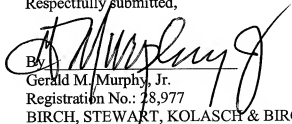
Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Paul D. Pyla, Reg. No. 59,228, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

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AUG 22 2008

Respectfully submitted,

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Enclosure: 37 C.F.R. § 1.132 Declaration of Mr. Hiei